

No. 24098-168

Appelbaum/Dul 9/12/93

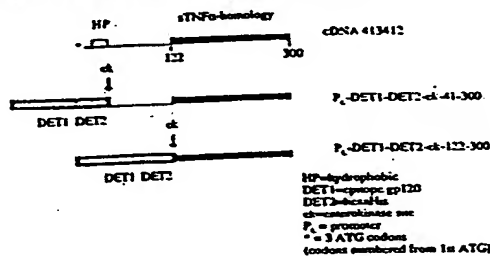
EXPRESSION OF TNF-LIKE PROTEIN
HGS clone 413412 = HTFAND8

Objectives:

- Express as fusion protein in E. coli—to be used for raising antibodies
- Express in soluble form in E. coli or other systems—to be used for receptor binding and activity assays

Accomplishments:

- Plasmid DNA from HGS strain (purified by K. B. Tan) was sequenced.
- The DNA sequence in the open reading frame agreed with that reported by HGS.
- There was a 1 bp difference upstream of the first of 3 closely spaced in-frame ATG codons, which makes it more likely that the first ATG is the translation initiator codon (TTCAATGG in HGS sequence is ATCAATGG in SB sequence; the latter is in good agreement with Kozak consensus upstream sequence)
- Two fusion constructs being made for E. coli expression; both have the potential for release of soluble protein following enterokinase digestion.



SEQUENCE of: TNF α human check: 6906 from: 1 to: 233
ID: TNF α human STANDARD: PRT: 233 AA.
AC: P01375
DT: 21-JUL-1986 (REL. 01. CREATED
DT: 21-JUL-1986 (REL. 01. LAST SEQUENCE UPDATE)
CT: 01-JUN-1994 (REL. 29. LAST AMOUNT/DATE)
CE: TUMOR NECROSIS FACTOR PRODUCTION (TNF- α) (CYCLODEXTRIN)
COT: 413412.Pap check: 5906 from: 1 to: 276
TRANSLATE of: 413412 check: 6736 from: 56 to: 3643
Generated symbols: 1 to: 530.
LOCUS: 413412
DEFINITION: Pox ligand (has mutation)
ACCESSION: 523412
LIBRARY: HTF human Pinctum Tumor
Symbol comparison table: GeneBank: (C)Gene, Data, Update) SeqComp.Cmp
CompCheck: 1234
Gap length: 7.000 Average match: 0.340
Length: 0.250 Average mismatch: -0.196
Quality: 75.0 Length: 233
Ratio: 0.333 Gaps: 2
Percent similarity: 33.394 Percent identity: 16.616
Tnf α human = 413412.Pap July 11, 1993 15:06 . .

5 GSHDVEACALPRTGQPCENCLFLSLFTLWATLPLUNG 54
60 AGLACFLKEDDTGNGCHDSEPCQVGLAQVHKLPTST 99
65 VICHQRETFPHLESLPLAQVSELENTFELRGEHPQKGL 103
70 LSTVQKQGLPLVLRGCPQVARTCTGCHDSTLESFVSDAL 147
75 LKLPVLRGCPQVARTCTGCHDSTLESFVSDAL 147
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SITE OF START
DET1-DET2 EK
122-300

SCIENTIST SIGNATURE

Edward J. Appelbaum

DATE

James A. Donald Feb 9 1994

SYNTHETIC DNA REQUEST FORM

Please read these instructions before you write your oligos:

1. Please write all oligo sequences from 5' to 3' direction.
2. Write sequences in capital letters using a pen (Not pencil).
3. You can request more than one oligo on a same page.
4. Write your name, project, department and phone number.
5. Write your sequences as 5' ATG - CCC - GAA - TTC - AGA - TTT 3'
6. If you need specialty oligos (Biotin, Amino, Uracil, Dendrocl, Thio-esterase etc., please write them accordingly).

OLIGO SEQUENCES:

1. 5' CGG - GAT - CCT - CGA - CGA - CGA - CAA - ACA - GCT -
GAA - GCA - GAT - GCA - GGA 3' SB 6686
2. 5' CCG - GAT - CCT - CGA - CGA - CGA - CCA - AAT - AGC - AGC -
TCA - CAT - AAT - TGG 3' SB 6687
3. ~~5' CCG - GAT - CCT - CGA - CGA - CGA - CCA - AAT - AGC - AGC -
TCA - CAT - AAT - TGG 3' SB 6687~~
4. 5' GCT - CTA - CAT - TAG - CCA - ALT - AAA - AAG - GLL -
SB 6688

Requestor: EDWARD DUL

Project: CHIMES

Department: GRS

Telephone Number: 7708

Indicate for what purpose you are requesting these oligos:

☒ PCR primers ☐ Sequencing primers ☐ Linkers ☐ Probes

Antisense

Gene construction

Affinity Columns

Hematology Clinical Center, Los Angeles Harbor Medical Center, Los Angeles, CA 90007

Please call 7708 for more information or help.

These oligos were designed
from the DNA sequence of
HGS clone 413412 (ATG-343)
provided by HGS

HGS clone 413412 (ATG 343) was sequenced in its
entirety and was in agreement with the HGS sequence.
This was the template for the PCR reaction of the TNF- β gene
product.

L1, L2 designate PCR reactions to obtain
DRT1, DRT2 & K 41-300

S1, S2 designate PCR reactions to obtain DRT1, DRT2
& K' 122-300

SCIENTIST SIGNATURE

Edward Dul

DATE

11/17/95

withers James O'Neill Feb 7, 1996

24098-170

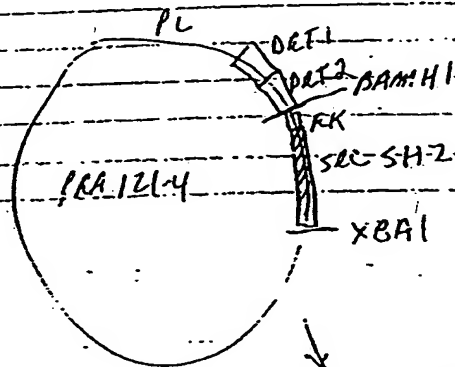
1.2 µl/50 µl H₂O

ATC 343-2 µl

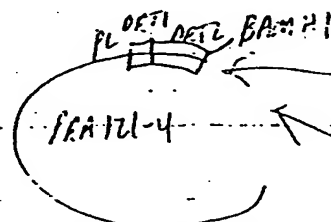
	ATC 343	ATC 343	ATC 343	ATC 343
SB 6696 (44-µl)	1.38 µl	95 µM		
SB 6697 (45-µl)	1.13 µl	107 µM		
SB 6698 (27-µl)	1.40 µl	157 µM		
ATC 343 (40-µl)	1.2 µl	1.2 µl	1.2 µl	1.2 µl
SB 6696	5 µl	5 µl	5 µl	5 µl
SB 6697	11 µl	11 µl	11 µl	11 µl
SB 6698	5 µl	5 µl	5 µl	5 µl
SB 6699	8 µl	8 µl	8 µl	8 µl
SB 6700	12 µl	12 µl	12 µl	12 µl
SB 6701	13 µl	13 µl	13 µl	13 µl
SB 6702	1 µl	1 µl	1 µl	1 µl

cut clone for fragments with BamHI/XbaI

Digest pRA12L-4 with BAMHI/XBAI



BAMHI/XBAI



ATC 343
L1 L2 and
ATC 343
S1 S2

cut with BAMHI/XBAI

H₂ (150 BP)

S1 (2500 BP)

ligate

SCIENTIST SIGNATURE

Richard Hef

DATE 11/17/95

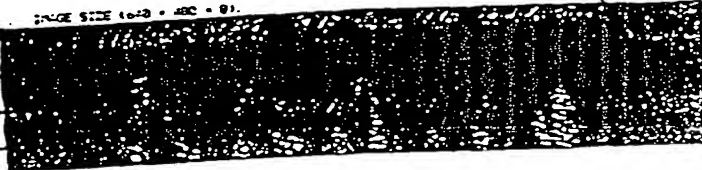
Witness: Peter A. Poulak Feb 9, 1996

24098-172

Wrote
cells

STORAGE ENCLAVE 10-16-95 14:00:00

IMAGE SIZE (x2) = 400 x 80



Run Western Blot of above sample using 178.1 (D.F.I. antisera)

Band on creamasee lights up using D.F.I. Antisera (178.1)

SCIENTIST SIGNATURE

Richard D. Hall

DATE 11/17/95

return to AT sample 527, 1996

INF-like gene (ATG-343) now called TL2-L (long)
and TL2-S (short)

Chris Jones purified a 1 liter induction of
TL2-L (Both DNA sequences of TL2 DRT1 DRT2
forms have been confirmed as expected)

Material was highly insoluble and C. fuel led to
solubility. It is in C. fuel and dilute it off

obtained ~ 30 mg material in 65 ml

Inject in rabbits for Antisera

To determine solubility ^{at low Temp} Transform DRT1 DRT2 & TL2
(Both forms L and S) in G198 with Tryptophan
induction at 28° and 32°

		0	4
1) G198	TL2-L1 (28°)	.460	1.19
2) G198	TL2-S2 (28°)	.436	1.07
4) G198	TL2-S2 (32°)	.407	1.16

whole cell pellets / sol/insol fractions

* Have obtained from HGS 2 Antisera to TL2 (ATG)
call HGS TL2-1 and HGS TL2-2

Obtained pre-blend from TL2 rabbits here (Both
pre-blend and 1st blend - called CK9A and CK9B

I was unable to see expression as well in this
strain as I was able to see with AR58 & AR170

Run Westerns (crude cells/100ul - sol/insol 30 ul
100ng DRT1 DRT2 TL2 purified by Chris Jones
1/1000 dilutions of RACK

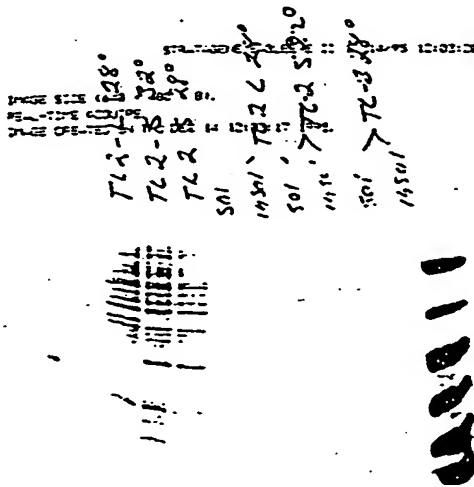
SCIENTIST SIGNATURE

DATE

Refused to sign

Antisera (Jove Abouille 2/11/96
Feb 9, 1996

Western Blot showed recognition of purified Tc2 with
 both my outer sera (CK9B) but much more faintly let val,
 with the HCS outer sera Tc2-2



5I698

Tc2

induction

cosmetic
STAIN

NH₂ Terminal of CKB-13 purified from *Phasophila*
 permits recognition of CKB-13 in cells (PMK)

SCIENTIST SIGNATURE

Edward H. Paul

DATE 2/1/96

written by J. Paul Feb 9, 1996

62199 induction of PEA DET1 DET2 TL2

overnight OD⁵⁵⁰ checked

L1-28°C - 3.5

S2-28°C - 3.1

L1-32°C - 4.8

S2-32°C - 4.6

Dilute Each ON in Induction media

	vol of n	Vol Ind media	OD ⁵⁵⁰ Diluted cells
L1-28°	1.5	25 ml	2.69
S2-28°	2	" "	2.21
L1-32°	1	" "	2.58
S2-32°	1	" "	2.44

1 hr 15 mins later

	2'	4' after addition of 100 µg/ml tetracycline
(1) L1-28°	530	1.97
(2) S2-28°	463	2.0
(3) L1-32°	542	2.26
(4) S2-32°	445	2.14

normalize whole cells in lysis Buffer

set aside cells for sonications - normalize them in Buffer A + lysozyme

inc - 30 min

sonicate

spin in microfuge - 30 min

SCIENTIST SIGNATURE

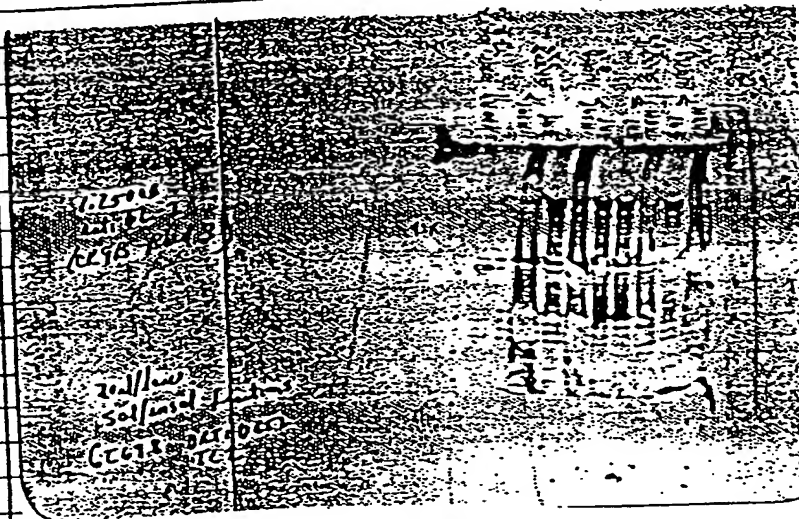
Richard P. Duf

DATE 2/8/96

Inters Jones at Pennell Feb 91 1996

Run 15% Precast gel of TL2 induction, (Coomassie whole cell
sol/in sol fractions Western Blot 20 μ /lane each sample

1: 2500 dilution TL2 antisera (CK9B Ab43)
1: 5000 dilution anti-Robust



gel is overloaded
and for Antisera is
not diluted enough.

Some encouraging results
(1) Antisera recognizes

(2) Antisera recognizes
short form of TL2
in supernatant at
28°C and possibly
32°C

This Western must be
re-run loading less sample and diluting primary antisera more.

Input RABBITs CK9A CK9B WUL DETIDITZ TL2 Long
input Robusts CK9A CK9B WUL CCA2 peptide

13 DO TRANSFECTION of MTALII CCA2 Long #5 into 52 cells

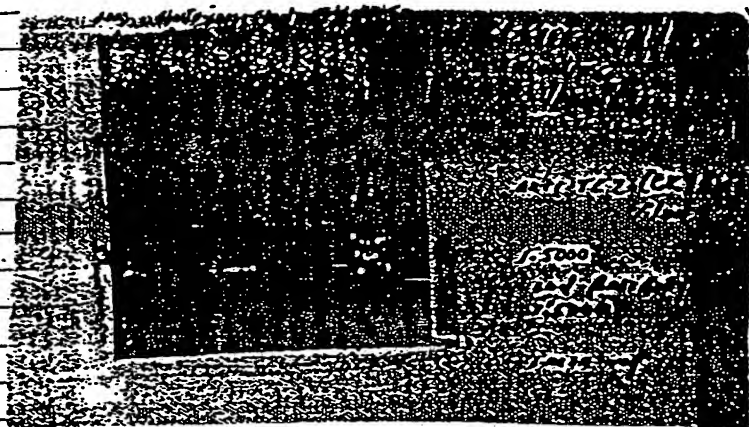
MTALII CCA2 Long #5	750 ng/1	25 μ
PC6 reagent	910 ng/1	1.5 μ

SCIENTIST SIGNATURE

Ed Huff

DATE 3/5/96

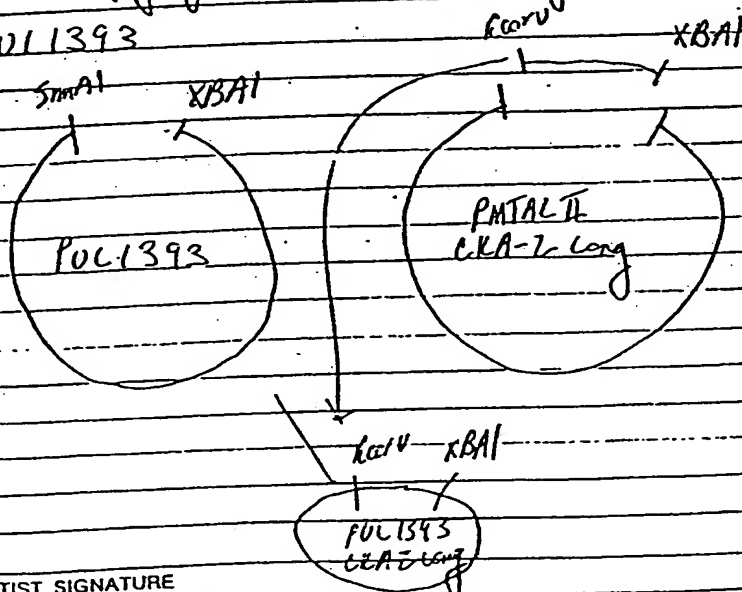
Re-run Western blot of TH2 induction using 4th lane sample and antisera at 1:5000 dilution



15 DNA sequence of PMTALII CKA-2 Long is AS expected

Strategy of CKA-2 Long Cloning is "Baculovirus Vector"

POL1393



SCIENTIST SIGNATURE

Kotkuf

DATE 5/5/96

No. 24095-196

yield of URAZ - long KLOU (YRA) must enter (1000/16)

323

Setup ligation

4 1393

4 1393

6 UAS long

4ul

PCR 1393 (0.5 µl/100 µl) 4ul

UAS long (0.5 µl/100 µl) 4ul

6ul

17ul

11ul

6ul

Sx. Bcl. I ligase

6ul

6ul

Ligase

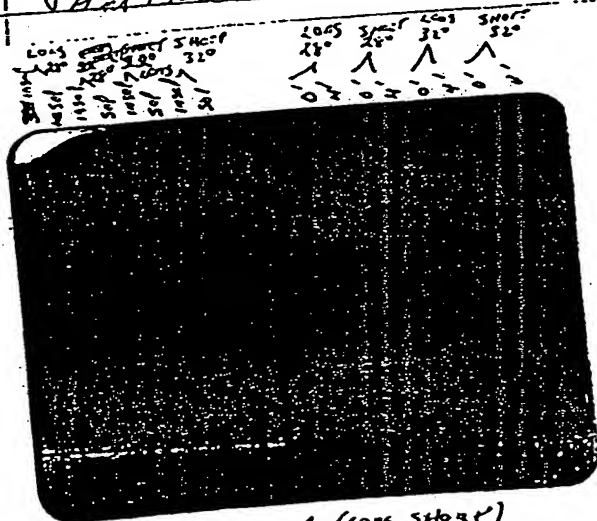
1ul

1ul

16°C 6/11

LB 25.25 Trans from in DH5α

Run agarose gel of salmonal fractions GE698 induction
of DET1 DET2 TC2 (Pg 191) Also replot Western using Pet 1
Antisera

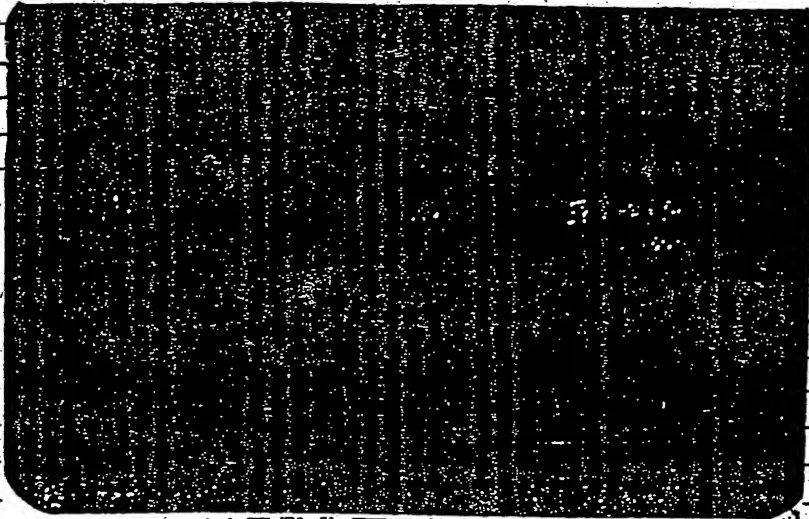


DET1 DET2 TC2 (long, short)
GE698 Induction

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[Signature]

DATE 3/5/9



2.6 Coomassie and Western Blot using Anti AAT1 showed solubility of AAT1 AAT2 TC2 slat

Do 1 liter induction of AAT1 AAT2 & TC2 (Long and Slat) for Sechrist's Purification

OD⁶⁰⁰ of overnights (28°C)
 TC2 - long - 2.2
 TC2 Slat - 2.15

(28°C) Dilute O/N 1:10 (50 ml / 450 ml induction medium)

OD⁶⁰⁰ at time of dilution

OD⁶⁰⁰ Addition of Tryptophan Harvest (g/L)

1 TC2 Slat (500ml)	.191	.410	2.0
2 " "	.237	.456	1.86
3 TC2 Long	.776	.404	1.82
4 " "	.734	.410	1.84

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E. J. [Signature]

DATE 3/5/96

No. 24098-198

3 28

DET1 DET2 TLE 49 scale (secal)

STRATOSPHERE EAGLETYPE 22 03/23/84 111131 61198

Long long short short

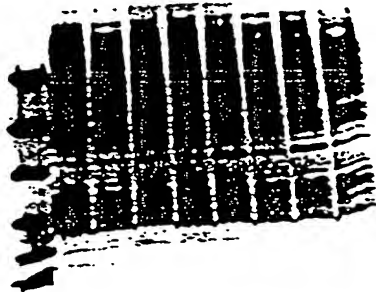
IMAGE SIZE (640 x 480 x 81)

INTEGRATE PERIOD IS 0.01 SECONDS ON 3 COUNTS

IMAGE CREATED ON SUN MAR 23 11:13:39 1984

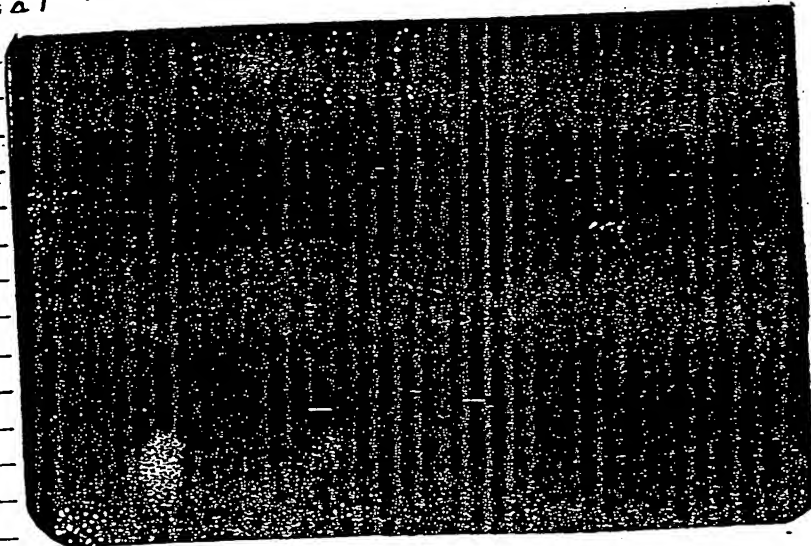
2800

0 4' 0 4' 0 4' 0 4' 0 4' 20 d/ful



Seawater above samples for sal/insal studies

Western Blot - Probe w/ul (Anti DET1 - 178.1) and (Anti TLE
CR901)



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Ad 1 ful

DATE 3/5/86

material (TL2-Slant/SLM) looks soluble with DAPI anti-sense
Turnover to SIB1/FISH

29 More colonies present on pHL393 LAR long than Vector
Please

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Ed. Puf

DATE

3/5/96

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